A Synopsis of thesis entitled

Studies on Biochemical and Histopathological Alterations Induced by Bacterial Pathogens On Selected Fresh Water Carp

For Registration of Ph.D.

In

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By

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Introduction

Human consumption of fish is increasing (from 100 to 110 million tonnes from 2002 to 2006). However, the tonnages fished have now reached their limits and the fish stocks are declining in some areas of the globe. This difference corresponds therefore to the development of aquaculture. The rapidly changing sector has in fact experienced a strong growth in recent years of around 10% per year. Global production fish culture reached 53 million tonnes in 2008 and today the majority of fish consumed worldwide comes from fish farming. The most carp, followed by tilapia, salmonids and siluriformes. While the sector is largely dominated by Asia, which produces 90% of the global volume of farmed fish.

However, the diseases that can be rampant in livestock farming and affecting the production of fish are a major obstacle to the development of the fish farming sector in India and in the world. Many pathologies can occur in fish farms. The pathogens most frequently reported by people who have performed field diagnoses in India in 2003 were bacteria (46%), viruses (20%), parasites (18%) and other etiological agents such as mycoses as well as pollution and nutritional diseases.

The main bacterial pathologies and their etiological agents reported in India are furunculosis (Aeromonas salmonicida), cold water flavobacteriosis (Flavobacterium psychrophilum), streptococcosis (Streptococcus iniae, Lactococcus garviae or Vagococcus salmoninarum), yersiniosis (Yersinia ruckeri) and flexibacteriosis (Tenacibaculum maritimum, formerly Flexibacter maritimus). This is not about prevalence but statements that depending on the importance given by professionals involved, may appear over-declared depending on emergencies and regulations or under-declared compared to their actual presence in fish farms. In addition, these statements can also be "distorted" by a lack of knowledge at the root of groupings under the same pathology of identical clinical signs due to agents different bacteria.

Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. The actual role of micro-organisms may vary from a primary pathogen to that of an opportunistic organism which renders its host moribund by initiating a disease process. Bacterial flora of fish is a direct reflection of its environment.

Analyzes carried out by field pathologists often go as far as isolation bacterial, primarily for performing antibiograms. These methods do not allow generally not diagnosing the presence of pathogens in carriers asymptomatic or eggs for example (in the case of vertical transmissions). It exists few tools available today to perform precise molecular diagnostics and reliable, especially for Flavobacterium psychrophilum flavobacteriosis. It's in the idea of responding to this need for generic knowledge about these bacteria, necessary to long term to help in effective management of the diagnosis and control of these diseases, which is part of the work of this thesis.

The question of the control and surveillance of pathologies is becoming increasingly of importance in the aquaculture sector. In this aim, the National Research Institute Agronomics (INRA) has been involved, for about thirty years, alongside fish farmers and aquaculture veterinarians to improve the diagnosis and control of the many pathologies affecting fish farms.

An exoenzyme or extracellular enzyme is an enzyme which is secreted by the cells and works outside of that cell. It is usually used for breaking down large molecules that would not be able to enter the cell otherwise. Bacteria secrete exoenzymes to hydrolize macromolecules into smaller molecules capable of being transported across the cytoplasmic membrane into the cytoplasm which is the site of most bacterial metabolism. Extracellular enzymes produced by bacteria are one of the major factors for their pathogenesis. Chitinase, lipase, protease and lecithinase are among these enzymes catalizing the process of infection. Some pathogenic bacteria are able to produce and secrete enzymes that compromise cell structure of the host and enable the bacteria to work their way further into the body. Interestingly, virulence factors also are secreted proteins or enzymes such as invasins, lecithinase and other common lipases and proteases (Secades and Guijarro, 1999; Lafferty, K. D. 2008).

Lipase is an exoenzyme that hydrolyzes lipids into fatty acids and glycerol acts as an important extra-cellular virulence factor affecting several immune system functions through free fatty acid generated by lipolytic activity in animals and humans. The amplification of small fragment of *up H3* gene was used to confirm *A. hydrophila* isolated from aquatic environment (Sharma et al., 2012;).

Proteases hydrolyze proteins into polypeptides and amino acid subunits. Microbial proteases have been proposed as virulence factors in a variety of diseases caused by microorganisms as they have the potential to destroy the structural and functional proteins that constitute host tissues as well as

important in host defense. Proteases play a crucial role in numerous pathologic processes as they have been linked to a number of degenerative diseases viz. arthritis, tumor invasion and metastasis. Damage of host tissues by degrading elastin, collagen and proteoglycan by *Pseudomonas aeruginosa* is partly done due to activity of alkaline protease and elastase (Sakata et al., 1980).

Review of Literature and Development in the subject (Previous work done in the relevant area):

Fish are a heterogeneous group of animals comprising more than 21 700 species (Fänge, 1994). Fish live in a challenging environment facing so many problems. In the aquatic environment, fish are in constant interaction with a wide range of pathogenic and non-pathogenic microorganisms (Subramanian *et al.,* 2007). In the aquatic environment, fish are in constant interaction with a wide range of pathogenic and non pathogenic microorganisms (Subramanian *et al.,* 2007). Microbes play an important role in affecting fish health. Fish suffer from various types of diseases. All fishes carry pathogens and parasites as normal flora, and if the pathogenic load increases it lead to disease. Disease is prime agent affecting fish mortality, especially when fish are young (Sharma *et al.,* 2012). Pathogens which can cause fish disease comprise viral infections, bacterial infections, fungal infections, and mould infections (Axelrod, 1989). Fishes in farms are susceptible to several bacterial infections mainly when reared in high density conditions. Disease outbreaks among fishes elevate the mortality rate and decrease the productivity leading to high economic loss to fish farmers (Sharma *et al.,* 2012). The prevalence of bacterial pathogens has been well documented in several cultured and wild fish water species (Moustafa *et al.,* 2010). Numerous candidates of antigen are now available to induce protective immune responses against opportunistic pathogens (Morin and Hopkins, 2002). The search for conserved protective antigens is an important element of this vaccination strategy because of the relatively large number of pathogens under consideration and the many serotypes, which might be clinically relevant (O Riodan and Lee, 2004). Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients contact with fish and related environment, dietary habits and immune system status of the exposed individual (Novotny *et al.,* 2004). Microbial investigation for characteristics of potential pathogenic microorganisms for fish will allow the application of adequate measures to prevent and control the main diseases limiting the production of fishes. *Aeromonas hydrophila* mainly causes motile aeromonad septicemia (MAS) and has also been reported to cause secondary infections associated with EUS outbreaks (Roberts,

1993). The disease caused by *A. hydrophila* has also been called Red-sore disease (Huizinga *et al.,* 1979). It does not usually cause problems in fish populations under normal conditions, but when fish are under environmental or physiological stress or infected by other pathogens (Fang *et al.,* 2000). Several studies have described a wide variation in the pathogenecity of *A. hydrophila* in different fish species (Sharma *et al.,* 2012). This is mainly due to the heterogenecity of strains and differences in the adhesive and enterotoxic mechanisms responsible for causing infection in fish (Fang *et al.,* 2004). Heuzenroeder *et al.,* (1999) showed that the mortality was dependent on the concentration of bacteria and the appearance of clinical signs in fish that eventually died of a major virulent factor, when its pathogenicity was studied using a suckling mice model infection. In the aquatic environment, fish are in constant interaction with wide range of pathogenic and non-pathogenic microorganisms (Subramanian *et al.,* 2007). Our emphasis in the present study is on the need for screening and isolation of bacterial and fungal from the infected fish sample and studied the LC50 values after the administration of the microorganism.

The primary purpose of the research activities conducted within this doctorate is was to study infections by protozoa and enteric microsporidia in species farmed fish and fish mycobacteriosis in farmed environments e wild.

Objectives of Research/ Proposed Hypothesis:

The research will be therefore developed along with following main lines: The present study will be undertaken with following objectives.

1) To evaluate the presence, spread and pathogenic effects of bacterial parasites associated with selected farmed fish species
2) To analyze the change in host fish by applying histological methods aimed at expanding knowledge on the etiopathogenesis of infections sustained by these pathogens
3) To define the correct diagnostic approach to be applied
4) To deepen the knowledge on fish bacteriosis in farmed fish with particular attention to the study various virulent factor associated with disease progression in selected fish hosts
5) to evaluate the alteration in various biochemical component of host n the selected farmed fish in the transmission of infection to aquaculture environments.

Methodology to be adopted:

i. Sample collection The fishes will be collected from various sources like fish farms, seed farms; cold storage, market etc. were brought from different districts of Marathwada, Maharashtra. These fish samples with visible disease symptoms and also on the surface of other body parts will be selected. After collection fish samples will be transported to the laboratory for further studies.

ii Determination of total viable count and isolation of bacteria from infected portions of fish The fish samples with infected portions having visible symptoms will be washed thoroughly with sterile distilled water. The infected portion will be swabbed and swab will be suspended in saline solution (0.1 % NaCl). Serial dilutions were made using saline solution and 10-5, 10-6 and 10-7 will be plated on nutrient agar (Hi media) to determine the average of number of colonies.

iii Identification of bacterial isolates based on morphological and biochemical characteristics The morphological, biochemical and molecular characteristics of bacteria isolated from diseased fish samples will be done following the method s described earlier (Collins et. al., 1989; Cappuccino and Sherman 2002). Biochemical characterization will be carried out as per Bergey's manual and was identified according to Bergey's Manual of Systematic Bacteriology (Kreig and Holt, 1984).

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v. Study of bacterial infection on physiology and nutritional status of infected fish. The infected fishes will be studied for the impact on various physiological process of fishes. The effect of infection on nutritional status of the fishes will be studied. The assessment of protein content, lipid contents, various vitamins etc..

Importance of study/ Society application:

International status

Since fish diseases pose a great threat on global fish culture, different countries and international organization have made effort in studying and monitoring the status of fish disease with a hope of finding ways to mitigate or control the problem of fish disease. The international organization, Office International des Epizooties (OIE), has undertaken to monitor the status of fish disease globally. OIE is a reference organization recognized by the World Trade Organization in achieving

veterinary services' compliance of participating countries. OIE published a manual for identifying fish diseases and imposing relevant treatments measures to ensure all participating countries have access to the latest techniques in treating fish diseases (Phylum, 2010). Moreover, OIE holds a vaccine bank to allow rapid supply of vaccine to infected countries to achieve eradication when there is outbreak of fish diseases (OIE, 2014). Besides international organizations, many countries also put a lot of effort in studying fish diseases. In India, Ireland and some Nordic countries also conducted surveillance programme to have a better understanding of the fish disease status in their countries (Hastein et al. , 2001; Marine Institute, 2014).

National Status

In Marathwada no systematic research on bacterial disease in fish has been carried out. Therefore the study of aquatic bacteria associated with fish is very limited in Marathwada region of Maharashtra. Our earlier work was a sincere attempt to assess the bacterial population in aquatic environment and their involvement in causing diseases in fish. Darak and Barde (2015) reported *Aeromonas* sp. and *Pseudomonas* sp. are very common bacteria associated with major carp and live fishes. The study was to identify the common bacterial pathogen associated with freshwater fish *Labeo calbasu* and characterize the pathogen in detail with their susceptibility to antibiotics for developing a control measure in future.

Significance of the study

Researchers need to constantly consider the effects that pathogens have on fish health. pathogens affect fish health through mechanical, physical and reproductive damage. These changes can reduce growth, fecundity and survival, change behavior and sexual characteristics, and result in many other maladaptive alterations of the infected host. These changes could have significant consequences at not only the individual level, but population, community and ecosystem levels as well. Research options are not limited, as there are existing criteria for both the selection of appropriate pathogens for analysis of fish stocks and the selection of hosts and pathogens as indicators of fish health and other stressors

Proposed work Plan/ Formulation and Structure of Study:

Year-wise Plan of work and targets to be achieved

First year:

1. Survey of the Marathwada region for the collection of various fishes and their diseases.

2. Isolation and maintenance of bacterial pathogens from the region

Second Year:

1. Characterization of isolated pathogens by Biochemical and molecular methods

2. Study of virulent factors associated with the isolated pathogens development

Third year:

1. Determination of histopathological alteration caused by the inoculation of the bacterial pathogen in laboratory and naturally occurring diseased fish samples

2. Assessment of alteration in various host component due to infection by bacterial pathogens

Fourth year:

1. Effect on the nutritional status of infected fish by bacterial pathogens

2. Analysis of data collected and verification of results

Fifth year:

1 Compilation of work and thesis compilation

References:

Axelrod, H.R., and D. Untergasser . Handbook of fish diseases. Neptune, NJ:TFH Publications, 1989.

Cappuccino JG and Sherman N (2002). Microbiology a laboratory manual (Vol. 1). New york: Benjamin Cumming.

Collins CH, Lyne PM and GM Grange (1989). Collins and Lyne Microbiological Methods, 6th Ed. Butterworth, London.

Darak O. S. and Barde R. D. (2015) Pseudomonas fluorescens associated with Bacterial Disease in Catla catla in Marathwada Region of Maharashtra. International Journal of Advanced Biotechnology and Research, 6(2)189-195.

Fang, H. M., Ge, R., and Y.M. Sin, 2004. Cloning, characterisation and expression of *Aeromonas hydrophila* major adhesin. Fish .Shellfish Immunol. 16(5): 645- 658.

Fang, H.M., Ling, K.C., and G.Y.M. Sin, 2000. Enhancement of protective immunity in blue gourami, Trichogaster tripchopterus (Pallas), against *Aeromonas hydrophila* and *Vibrio anguillarium* by *Aeromonas hydrophila* major adhesion. J. Fsh Dis. 23: 137-145.

Fänge, R., 1994. Blood cells, haemopoiesis and lymphomyeloid tissues in fish. Fish.Shellfish Immunol. *4*(6): 405- 411

Hastein, T., Hellstrom, A., Jonsson, G., Olesen, N. J., & Rimaila-Parnanen, E. (2001). Surveillance of fish diseases in the Nordic countries. *Acta Veterinaria Scandinavica Supplementum, 94*, 43-50.

Heuzenroender, M.W., Wong, C.Y.F and R.L.P.Flower, 1999. Distribution of two hemolytic toxin genes in clinical and environmental isolates of *Aeromonas* species:correlation with virulence in a suckling mouse model. FEMS Microbiol. Lett. 174: 131-136.

Huizinga, H.W., Esch, G.W., and T.C.Hazen, 1979. Histopathology of red-sore disease(*Aeromonas hydrophila*) in naturally and experimentally infected largemouth bass Micropterus salmoides (Lacepede). J. Fish Dis. 2: 263-277.

Kreig, N. R. and J. G. Holt, (1984). *Bergey's Manual of Systematic Bacteriology*. Volume-1. Williams and Wilkins. 964pp.

Lafferty, K. D. 2008. Ecosystem consequences of fish parasites. Journal of Fish Biology 73:2083-2093.

Marine Institute. (2014). Disease surveillance programmes. Retrieved 25 Jun, 2014, from http://www.marine.ie/home/services/operational/fishhealth/Disease_ Surveillance.htm

Morin, M.D., and Hopkins, W.J. 2002. Identification of virulence genes in uropathogenic *Escherichia coli* by multiplex polymerase chain reaction and their association with infectivity in mice. J.Urol. 60(3) : 537 541.

Moustafa M, Laila A, Mohamed MA, Mahmoud WS, Soliman and MY Elgendy, 2010. Bacterial infections affecting Marine fishes in Egypt. J. American Sci. 6(11):603-612.

Novotny, L., Dvorska, L., Lorencova, A., Beran, V and I. Pavlik,2004. Fish: a potential source of bacterial pathogens for humanbeings, Vet.Med.-Czech, 49(9): 343-358.

O Riordan, K., and Lee, J.C., 2004. *Staphylococcus aureus* capsular polysaccharides. Clinical Microbial. Rev. 17(1), 218-234.

OIE. (2014). Vaccine bank. Retrieved 25 Jun, 2014, from http://www.oie.int/ support-to-oie-members/vaccine-bank/

Phylum. (2010). OIE study: "Listing and categorisation of priority animal diseases, including those transmissible to humans"- Methodological manual.

Roberts, R.J., 1993. Motile Aeromonad septicaemia. In: Bacterial diseases of fish (Ed. By Inglis V., Roberts, R.J. and Bromage N.R), Blackwell scientific publications, Oxford, 143-155.

Sakata, T., Okabayashi, J. and Kakimoto, D. '(1980a). Variations in the intestinal microflora of tilapia reared in fresh and seawater. Bulletin of the Japanese Society for Scientific Fisheries, 1+6, 313-317.

Secades P, Guijarro JA (1999). Psychrotrophic proteolytic bacteria from cold environment of Gangotri glacier, Western Himalaya, India. Appl. Environ. Microbiol., 65(9): 3969-3975.

Sharma, M., Shrivastav, A.B., Sahni, Y.P., and G. Pandey, 2012. Overviews of the treatment and control of common fish diseases, Inter. Res. J. Pharma. 3(7): 123-127.

Subramanian, S., MacKinnnon, S.L., and Ross, N.W., 2007. A comparative study on innate immune parameters in the epidermal mucous of various fish species. Comparitive Biochemistry and physiology Part B: biochemistry and Molecular Biology. 148(3): 256-263.

Thampuran, N., Surendran, P. K., Mukundan, M. K., and Gopakumar, K. 1995. Bacteriological studies on fish affected by epizootic ulcerative syndrome (EUS) in Kerala, India.

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